BACKGROUND

- The London Patient was treated for refractory Hodgkin Lymphoma (stage IVb) with single HSCT with CCR5Δ32/Δ32 donor cells in 2016[1].
- We reported remission 18 months after analytical treatment interruption using measurements in blood only.
- Here we present data from other tissues and longer follow up.

METHODS

Droplet Digital PCR

We quantified HIV DNA using droplet digital PCR (ddPCR, Bio-Rad) targeting the LTR, gag and integrase region and shown as target copies per million cells tested[6]. RNaseP, a human gene which is present twice in diploid cells, was measured in duplicate to determine the input cell number. In all ddPCR runs, water and donor PBMCs were tested in duplicate as negative controls for the HIV target regions and U1 cells were tested as a positive control. Samples generating a single positive droplet were interpreted as negative based on the occurrence of a sporadic positive droplet in the negative controls (1/40 reactions). We further analyzed samples demonstrating ≥ 2 positive droplets in an intact proviral DNA assay (IPDA) essentially as described[7]. The IPDA assay is based on a duplex ddPCR targeting two regions in the viral genome that are present in most intact proviruses: the HIV packaging signal (Ψ) and the Rev Responsive Element (RRE) in Envelope (*env*).

Quantitative real time PCR

For each qPCR standards were tested in triplicate, in addition to 2 positive controls in triplicate and 6 negative control wells. 25000 cells worth of DNA (based on an Albumin qPCR) were used per well

HIV-1 antibody responses

Specific HIV-1 antibodies in longitudinal sera samples diluted 1:2 were tested in a qualitative western blot assay (New Lav Blot I, Bio-Rad). Standard and low sensitive (LS) versions of the Vitros anti-HIV-1 assay (Ortho-Clinical Diagnostics) and the limiting avidity antigen assay were measured in same samples

HIV-1, CMV and EBV specific CD4 and CD8 T cell responses

For intracellular cytokine staining (ICS) and peptide stimulation, PBMCs were thawed and resuspended in RPMI complete media. Following, overnight rest at 37^{0}C and 5%CO2, PBMCs were stimulated for 6h with 2μg/ml HIV-1 Gag pools or cytomegalovirus (CMV) pp65 (JPT Peptide Technologies) or the PepTivator® EBV Consensus pool containing 43 peptides of 8-20 aa in length

Mathematical Modelling

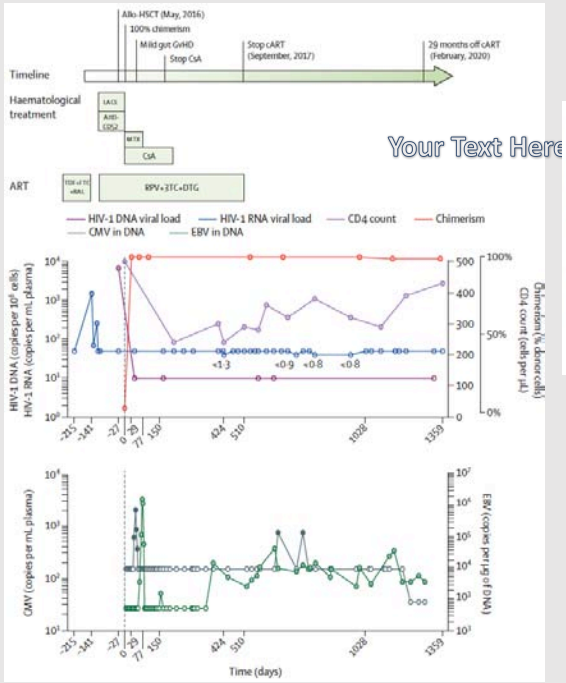
We predicted the probability of rebound using a previously-developed mathematical model and inference approach

RESULTS i

- T cell chimerism maintained at 99%
- pVL below 1 copy/ml to 29 months
- CD4 count 430 at 29 months
- EBV reactivation – see figure 1
- CSF viral load <12 copies/ml at 25 months post ATI
- CSF cellular HIV DNA negative at 25 months post ATI
- Semen plasma <12 copies/ml at month 21 post ATI
- Semen cellular HIV DNA negative at 21 months post ATI

The London Patient has been in remission for 30 months post ART interruption with evidence of low level HIV-1 DNA 'fossils' in peripheral memory T cells and Lymph node.

Figure 1: clinical course



RESULTS: Immune responses (Figure 2)
No CD4 or CD8 responses to HIV whilst CMV responses were observed. Note EBV reactivation in Fig 1

Figure 2. CMV and HIV-1 specific T cell responses

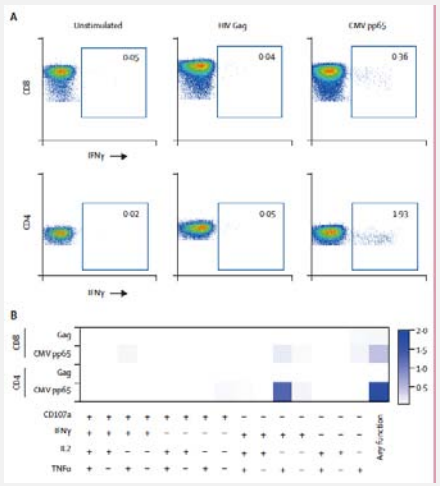
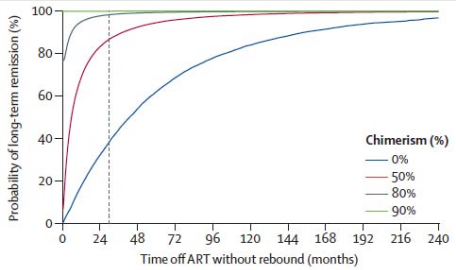


Figure 3. Predictions from mathematical modelling given no rebound at 29 months



RESULTS from tissue (table 1)

- Gut tissue negative by ddPCR
- Lymph node ddPCR low positive for env and LTR
- IPDA negative by ddPCR and qPCR
- Lymph node qPCR low positive for env and psi
- Peripheral CD4 memory low positive LTR and env

RESULTS (Figure 3)

Mathematical modelling (Figure 3):

If chimerism is >80% then >90% probability of cure

If chimerism is >90% then >99% probability of cure

Table 1: ddPCR on tissue compartments

Lymph node (27 months post ATI)

	Sample	Target	Replicates tested (n)	Cells tested (n)	Copies/million cells	Clinical interpretation*
ddPCR	Lymph node	LTR	14	2068220	33.6	Positive
	Water	LTR	4	0	-	
	PBMC	LTR	4	191290	<5.1	
	U1	LTR	4	3091	3409253	
	Lymph node	Integrase	8	1181840	<0.9	Negative
	Water	Integrase	2	0	-	
	PBMC	Integrase	2	97240	<10.3	
	U1	Integrase	2	1540	1720000	
	Lymph node	GAG	10	1349700	5.1	Positive
	Water	GAG	2	0	-	
	PBMC	GAG	2	96177	<10.4	
	U1	GAG	2	1613	1391197	
IPDA	Lymph node	PSI	14	2068220	1.6	Negative
	Water	PSI	4		-	
	PBMC	PSI	4	236720	<4.2	
	U1	PSI	4	2860	2289161	
	Lymph node	ENV	14	2068220	26.1	Positive
	Water	ENV	4	0	-	
	PBMC	ENV	4	236720	<4.2	
	U1	ENV	4	2860	2313986	
	No double positive droplet in IPDA (PSI+ENV+) SHEARING INDEX 0.39					

ATI = analytical treatment interruption
ddPCR = Digital Droplet PCR
IPDA = intact proviral DNA assay
* = based on ≤1 positive droplet per assay

Gut biopsies (22 months post ATI)

	Sample	Target	Replicates tested (n)	Cells tested (n)	Copies/million cells	Clinical interpretation*
ddPCR	Ileum	LTR	4	228580	6.7	Negative
	Caecum	LTR	4	358600	<2.8	Negative
	Rectum	LTR	4	151800	<6.6	Negative
	Water	LTR	2	0	-	
	PBMC	LTR	2	94050	<10.6	
	U1	LTR	2	1760	2800000	
	Ileum	Integrase	4	201080	<5.0	Negative
	Caecum	Integrase	4	326700	5.4	Negative
	Rectum	Integrase	4	136400	11.3	Negative
	Water	Integrase	2	0	-	
	PBMC	Integrase	2	97240	<10.3	
	U1	Integrase	2	1540	1720000	

ATI = analytical treatment interruption
ddPCR = Digital Droplet PCR
* = based on ≤1 positive droplet per assay

CD4$^+$ T-cells (28 months post ATI)

	Sample	Target	Replicates tested (n)	Cells tested (n)	Copies/million cells	Clinical interpretation*
ddPCR	CD4 cells	LTR	8	424160	<2.4	Negative
	T naive	LTR	8	282920	<3.5	Negative
	T memory	LTR	8	524920	6.7	Positive
	Water	LTR	4	0	-	
	PBMC	LTR	2	86790	<11.5	
	U1	LTR	2	1562	2887324	
IPDA	T memory	PSI	6	286770	21.5	Positive
	Water	PSI	6	0	-	
	PBMC	PSI	2	63690	<15.7	
	U1	PSI	2	3542	2335404	
	T memory	ENV	6	886380	6.9	Negative
	Water	ENV	6	0	-	
	PBMC	ENV	2	63690	<15.7	
	U1	ENV	2	3542	234826	
	One double positive droplet in IPDA (PSI+ENV+) SHEARING INDEX 0.24					

ATI = analytical treatment interruption
ddPCR = Digital Droplet PCR
IPDA = intact proviral DNA assay
* = based on ≤1 positive droplet per assay

CONCLUSIONS

- 30 month remission post ATI
- Negative HIV DNA/RNA results from CSF and semen
- Low level HIV-1 DNA in lymph node and CD4 Tmemory
- Absent HIV specific T cell immune responses
- Cure highly likely (Figure 3)

References:
[1] Gupta et al, Nature 2019

Table 1. Participant characteristics

Characteristics	Efavirenz Arm (n=293)	Dolutegravir Arms (n=587)	P-value
Female sex, n (%)	169 (58%)	362 (62%)	0.25
Age, median (IQR)	32 (27-37)	32 (27-37)	0.99
Married, n (%)	64 (22%)	114 (19%)	0.41
Tertiary or higher education, n (%)	21 (7%)	56 (10%)	0.23
Employed, n (%)	178 (61%)	365 (63%)	0.55
Baseline CD4, n (%)			
<200 cells/uL	87 (30%)	187 (32%)	0.69
201-350 cells/uL	89 (30%)	176 (30%)	
351-500 cells/uL	62 (21%)	106 (18%)	
>500 cells/uL	55 (19%)	118 (20%)	
Baseline viral load, n (%)			
<10k copies/ml	100 (34%)	186 (32%)	0.40
10k-100k copies/ml	124 (42%)	276 (47%)	
>100k copies/ml	69 (24%)	124 (21%)	
Pre-treatment drug resistance, n (%)*	**25 (9%)**	**98 (17%)**	**0.001**

Table 2. Univariable and multivariable regression of predictors of primary outcome

	Univariable Models		Multivariable Model	
	OR (95% CI)	*P*-value	AOR (95% CI)	*P*-value
Female sex	0.85 (0.61- 1.19)	0.34	0.83 (0.55-1.26)	0.38
Age (per year)	1.05 (1.03-1.07)	<0.001	1.03 (1.00-1.06)	0.05
Married	1.61 (1.03-2.51)	0.04	1.09 (0.65-1.83)	0.74
Tertiary education	1.03 (0.58-1.84)	0.92	0.83 (0.44-1.60)	0.58
Employed	2.02 (1.46-2.82)	<0.001	1.62 (1.09-2.42)	0.017
Baseline CD4				
<200 cells/uL	REF			
201-350 cells/uL	1.20 (0.80-1.81)	0.38	1.12 (0.72-1.96)	0.50
351-500 cells/uL	1.21 (0.76-1.91)	0.43	0.91 (0.51-1.63)	0.76
>500 cells/uL	1.20 (0.76-1.90)	0.43	1.01 (0.56-1.84)	0.96
Baseline viral load				
<10k copies/ml	REF			
10k-100k copies/ml	0.56 (0.38-0.84)	0.005	0.50 (0.30-0.83)	0.008
>100k copies/ml	**0.48 (0.30-0.75)**	**0.002**	**0.35 (0.19-0.64)**	**0.001**
High adherence	**0.34 (0.24-0.47)**	**<0.001**	**0.34 (0.23-0.50)**	**<0.001**
Study Arm				
EFV	REF			
DTG	0.71 (0.49-1.01)	0.056	0.88 (0.56-1.39)	0.59
Arm*WHO PDR	--		1.82 (0.61-5.42)	0.28

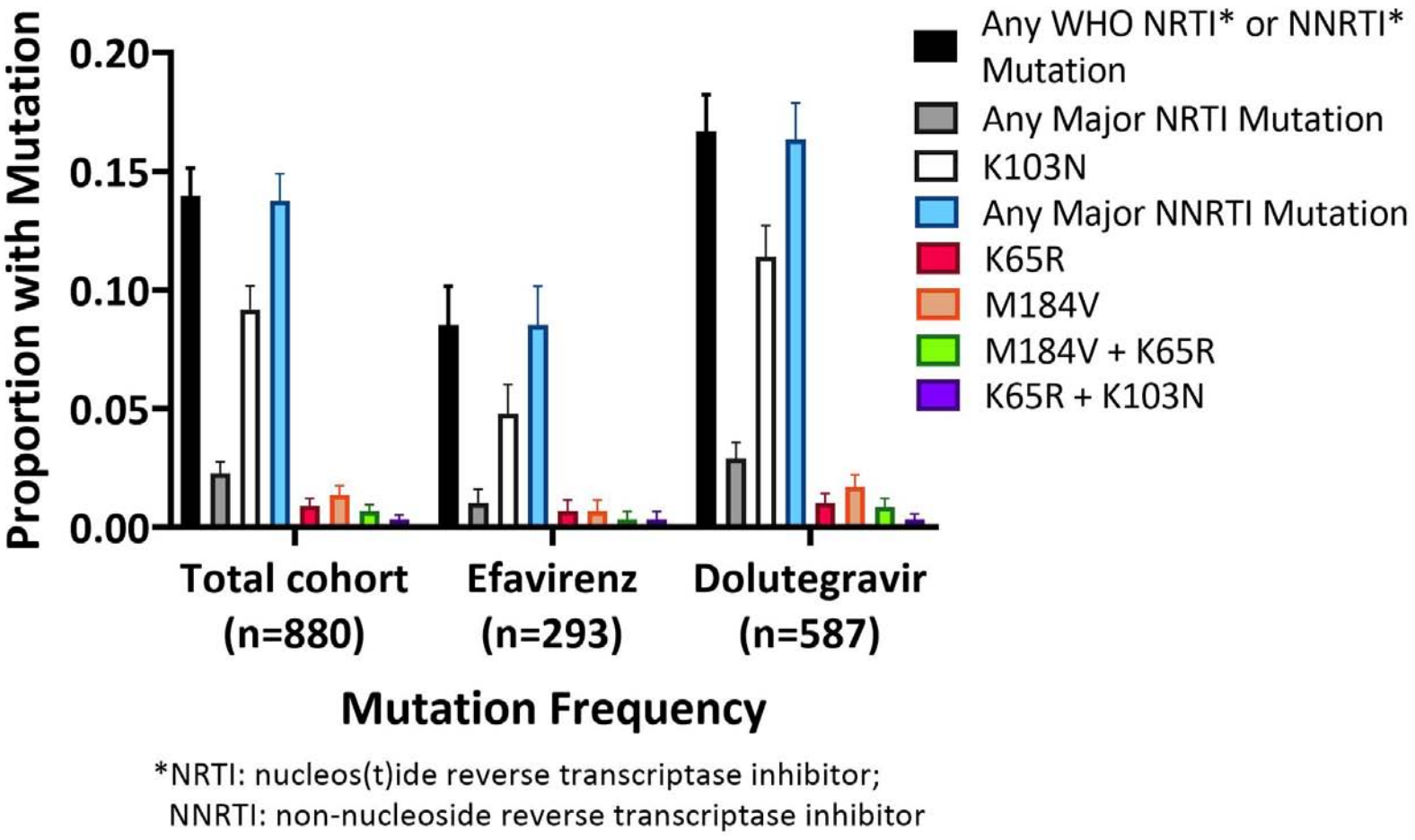


Figure 1. Prevalence of pre-treatment resistance in ADVANCE study (n=880)

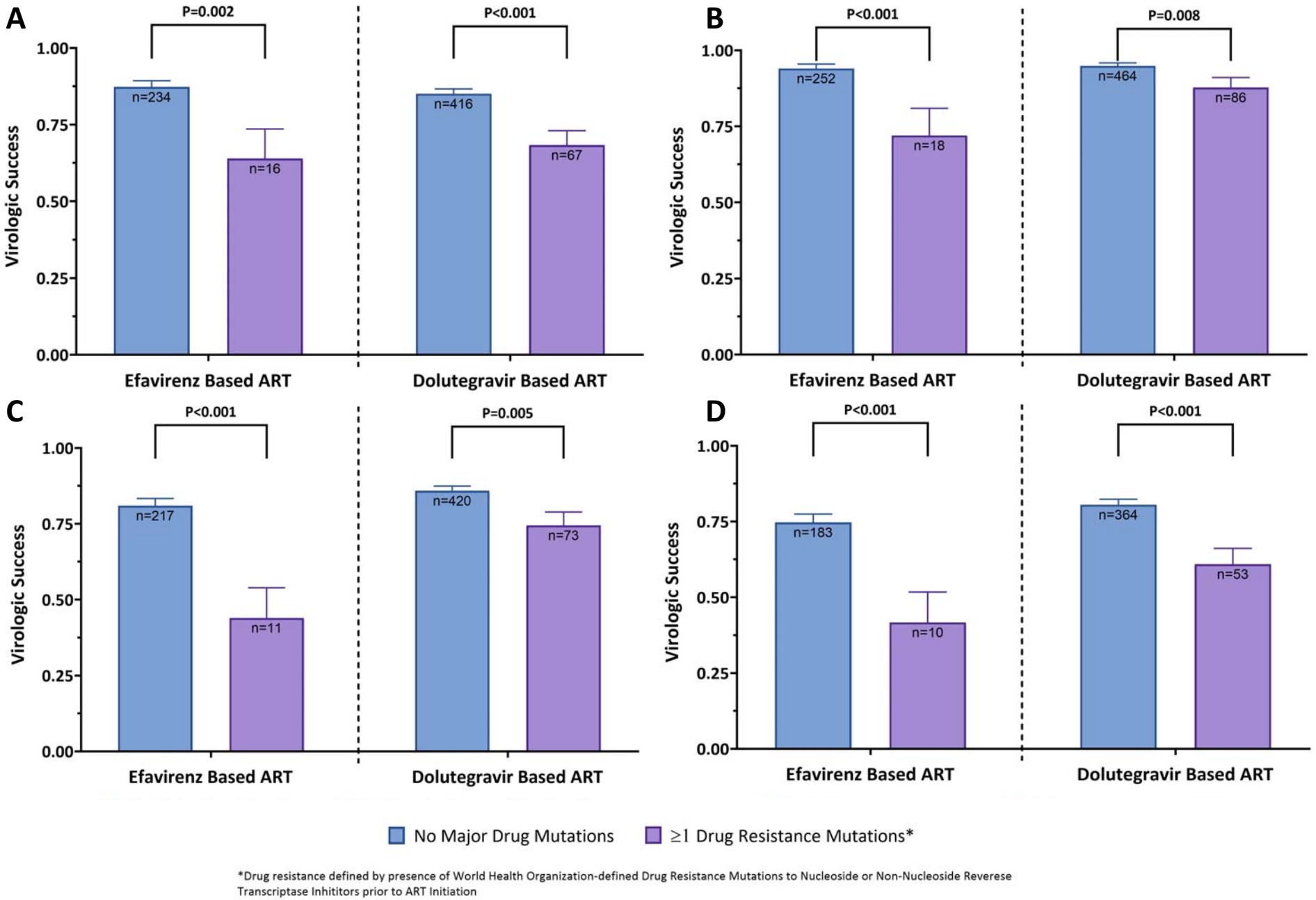
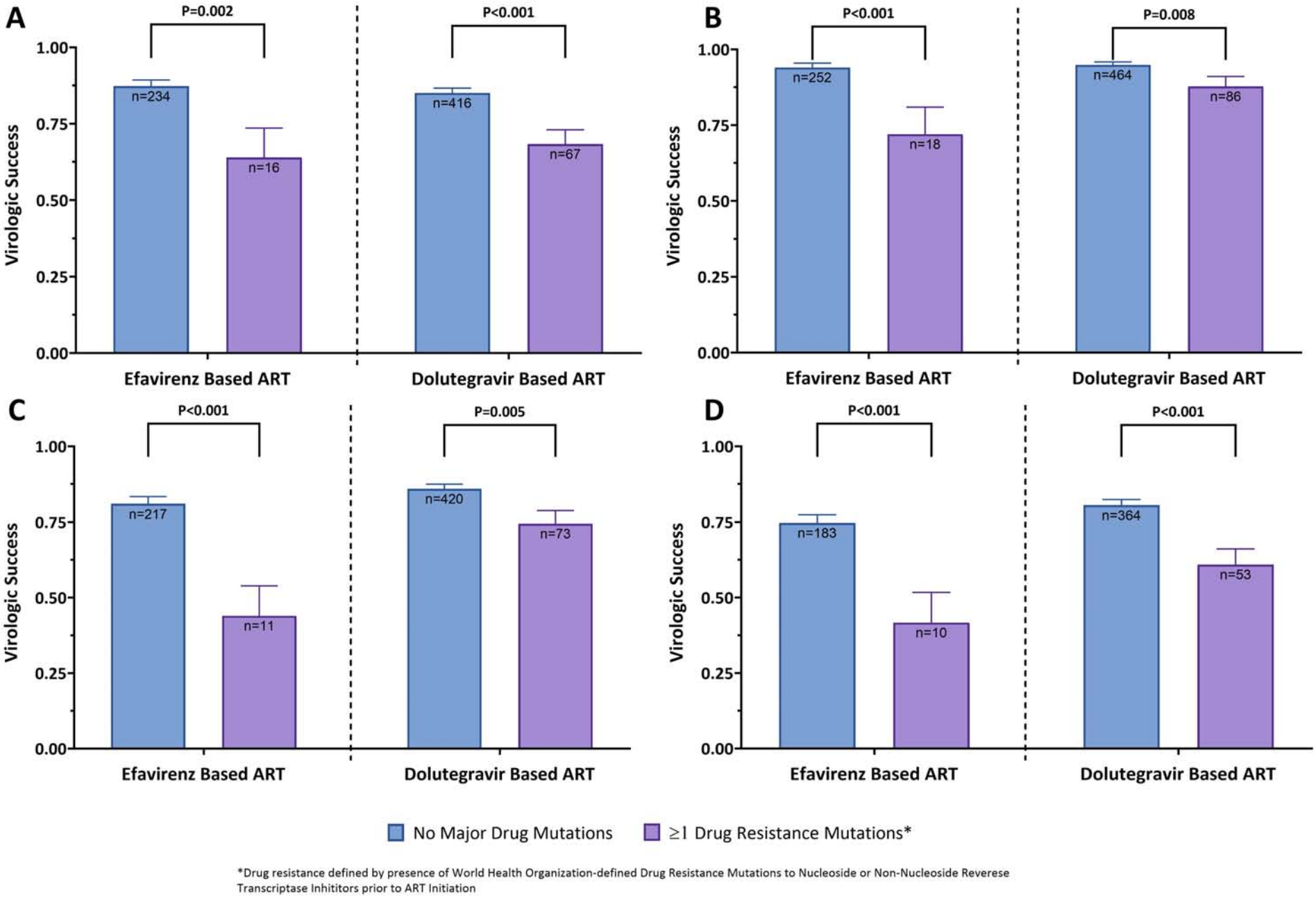


Figure 2. Primary (A) and Secondary (B) Virologic Outcomes and FDA 48-week (C) and 96-week (D) Snapshot Virologic Suppression by Treatment Regimen and Presence or Absence of WHO Pre-treatment Drug Resistance



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